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Symposium Abstracts

CHOLESTEROL AND ALZHEIMER'S DISEASE

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Symposium web site: <http://www.biozentrum.uni-frankfurt.de/Pharmakologie/BCS/cholesterolandalzheimer@neurobiologyoflipids.org> (also see abstracts for presenting authors' email contacts)

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July 26, 2002 featured the conference "Cholesterol and Alzheimer's disease" held at the Biocenter, Frankfurt University, Frankfurt/Maine, Germany. The conference program included ten lectures and eight poster presentations on different aspects of the possible role for cholesterol in Alzheimer's disease and cholesterol neurobiology. This truly international round table event did not aim to come to the consensus but rather to summarize the advances and to discuss directions for near future development. This article presents the abstracts and related bibliography and aims to introduce readers to the multifarious subject of neural cholesterol with special emphasis on Alzheimer's disease and related disorders.

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ORAL PRESENTATIONS

Lecture 1

CHOLESTEROL TRAFFICKING IN THE GOLGI COMPLEX AND AMYLOID BETA-PEPTIDE1-42

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Amyloid beta-peptide (A β) is thought to be one of the primary factors causing neurodegeneration in Alzheimer's disease (AD). This peptide is an amphipathic molecule that perturbs membranes, induces formation of reactive oxygen species, binds lipids and alters cell function. Amyloid β peptide, the main component of neuritic plaques seen in brains of AD patients interacts with cholesterol. This interaction is reciprocal. Cholesterol levels modulate amyloid precursor protein (APP) and A β synthesis. Conversely, A β alters cholesterol dynamics. Cholesterol transport and intracellular cholesterol distribution are altered by A β . The Golgi complex plays an integral role in regulation of cholesterol transport and distribution and data will be presented demonstrating that A β alters cholesterol distribution in the Golgi complex of astrocytes and neurons. Furthermore, effects of A β on cholesterol were dependent on peptide structure. Mechanisms of effects of A β on the Golgi complex may involve direct binding of cholesterol to A β and inhibition of PC-phospholipase C (PC-PLC) and PC-phospholipase D (PC-PLD) and data in support of this hypothesis will be presented. Both PC-PLC and PC-PLD contribute to regulation of cholesterol

transport. A β -induced modification of Golgi cholesterol content could impact on important Golgi functions such as protein sorting, sphingomyelin synthesis and assembling and release of lipid rafts that could disrupt cell function and membrane structure.

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Lecture 2

CHOLESTEROL AND NEURONAL FUNCTION

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We experimentally modelled neuronal cholesterol disbalance by acute biochemical increase of the turnover of cholesterol in rat hippocampal slices. Such an experimental set up impairs the redistribution of cholesterol from one cell to another via lipoprotein transport. While increasing cholesterol removal or immediately thereafter, we evoked and recorded two brain waveforms, paired pulse facilitation (PPF) or long-term potentiation (LTP), which are indicative of neurotransmission and synaptic plasticity, respectively. We found that the lack of cholesterol supply to neurons impaired PPF and LTP. From additional immunofluorescent analysis of the slices, we demonstrated that cholesterol disbalance also caused neurodegeneration of hippocampal neural cell processes and the appearance of tau protein pathology in the mossy fibers. We also analysed rats fed a cholesterol diet and discovered that they have increased hippocampal cholesterol biosynthesis and impaired LTP. Cholesterol fed rats were also characterized by Alzheimer's-like brain amyloid that we did not observe in the model of acute cholesterol disbalance.

Our data and research by others suggest that cholesterol homeostasis biological misregulation itself has a key role for synaptic plasticity impairment, neuronal degeneration and is the primary cause for several Alzheimer's disease hallmarks not limited to brain amyloid. Moreover, Alzheimer's changes in neurochemistry of amyloid beta, tau, neuronal cytoskeleton, and oxidative stress reactions likely represent physiological transitory mechanisms aiming to compensate impaired brain cholesterol dynamics and/or

associated neurotransmission and synaptic plasticity failure.

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Lecture 3

CHOLESTEROL AND TAU PROTEIN

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The fatal autosomal recessive neurovisceral lipid storage disorder Niemann Pick C (NPC) is a juvenile dementia with massive nerve cell loss and cytoskeletal abnormalities in cerebral neurons. These abnormalities consist of tangles of the otherwise highly soluble microtubule-stabilizing protein tau. Immunologically and ultrastructurally similar tangles are seen some decades later in patients with Alzheimer's disease (AD). We will provide evidence that tangle-bearing cells in both diseases show higher levels of free (i.e. filipin-positive) cholesterol than adjacent tangle-free nerve cells. The cholesterol accumulates either in a more diffuse way (mainly in AD) or in granule-like accumulations (mainly in NPC). In NPC, the neuron's cholesterol may origin from sources other than the alimentary tract. Experiments with a NPC mouse model revealed that even in pure neuron cultures the NPC-/- neurons accumulate free cholesterol in contrast to NPC-wt littermates suggesting that the cholesterol is either synthesized by the neurons or liberated from degenerated ones before it is taken-up by endosomal/lysosomal pathway. The accumulation of free cholesterol in the somata of NPC neurons is associated with a

decrease of cholesterol levels in myelin sheaths. In terms of tau protein, the NPC^{-/-} mice exhibit higher levels of AT8-positive tau, suggesting that the phosphorylation-dependent mAb AT8 has detected a tau-epitope in a state considered to represent early stages of tangle formation. Concomitantly to the increase in free intracellular cholesterol the message of the rate-limiting enzyme in cholesterol and isoprenoid biosynthesis, i.e. the HMG-CoA reductase, was found significantly reduced. Experimental blockade of the enzyme's activity by application of the lipid lowering drug lovastatin showed subcellular shifts in tau phosphorylation as monitored with mAbs AT8, 12E8 and others. In summary, our data showed interesting similarities between NPC and AD.

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Lecture 4

CHOLESTEROL AND AMYLOID BETA AGGREGATION

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One of the fundamental questions about the pathogenesis of Alzheimer's disease (AD) is how the soluble, nontoxic form of Abeta is converted to its insoluble, toxic form. We previously identified a novel Abeta species that strongly binds to GM1 ganglioside in human brains which exhibit early pathological changes of AD. Based on its unique molecular characteristics including its high aggregation potential and altered immunoreactivity, we hypothesized that amyloid β (A β) undergoes conformational alteration through its binding to GM1 ganglioside and acts as a seed for A β fibrillogenesis. In regard to the formation of GM1-ganglioside-bound A β , we recently reported that an

increase in the cholesterol concentration of the host membranes markedly accelerates the binding of A β to GM1 ganglioside. We then investigated whether the cholesterol concentration of cellular membranes could be altered under biological conditions that are associated with the risk factors for the development of AD. Since Wood and his coworkers previously reported that the cholesterol concentration in the exofacial leaflet of synaptic plasma membranes (SPMs) increases with age, we attempted to determine the distribution of cholesterol in the SPMs of the human apolipoprotein E (apoE)-knock-in mouse. We found that the cholesterol concentration in the exofacial leaflet of SPMs of the apoE4-knock-in mouse was approximately twofold higher than that of the apoE3-knock-in mouse. The results of our studies suggest that an increase in cholesterol concentration of the membrane accelerates not only the generation of A β but also the aggregation of Abeta through the formation of a seed for A β fibrillogenesis.

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Lecture 5

CHOLESTEROL, A MODULATOR OF MEMBRANE ASSOCIATED A β -FIBRILLOGENESIS

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One of the major pathological features of Alzheimer's disease is the presence of extracellular amyloid plaques that are predominantly composed of the amyloid- β peptide (A β). Characterization of

plaques demonstrated the predominance of two peptides differing at the carboxyl terminus by 2 hydrophobic amino acids, A β 40 and A β 42. Diffuse plaques associated with AD are composed predominantly of A β 42, whereas senile plaques contain both A β 40 and A β 42. Recently, it has been suggested that diffuse plaque formation is initiated as a plasma membrane bound A β species and that A β 42 is the critical component. In order to investigate this hypothesis, we have examined A β 40/42-lipid interactions using in situ atomic force microscopy, electron microscopy and fluorescence anisotropy. While the association of A β 42 with planar bilayers resulted in peptide aggregation but no fibre formation, this was not the case for A β 40 where we observed preferential fibre formation. Cholesterol, a key membrane component and modulating factor in AD, is inversely correlated with the extent of A β 40/42-bilayer interaction. These results were confirmed using fluorescence anisotropy to evaluate the effect of A β on membrane fluidity and fluorimetry to confirm membrane integrity. Our results suggest that the enhanced amyloidogenic properties of A β 42 are not correlated with fibril formation but aggregation on bilayer surfaces.

Lecture 6

MEMBRANE CHOLESTEROL AND A β

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Regarding Alzheimer's Disease (AD) membrane cholesterol seems to be involved in different steps leading to pathological events in the brain. First evidence that cholesterol has impacts towards the effects of A β on neuronal membranes became obvious when we correlated the effects of A β with the individual cholesterol content of human hippocampal membrane samples from AD patients and controls. We observed a negative correlation between the membrane disruptive effects of A β and the cholesterol content. Thus low levels of membrane cholesterol amplify the interaction of A β on brain membranes. These findings further implicate that cholesterol is involved in brain membrane alterations occurring during AD. It is remarkable that these disease related changes in cholesterol metabolism must be subtle and restricted on defined membrane pools, because total

membrane cholesterol content is not altered. To further evaluate the importance of membrane cholesterol for the effects of A β we modulated the content of cholesterol in synaptosomal plasma membranes (SPM) from the brain of mice. As an experimental approach we depleted cholesterol from SPM using methyl- β -cyclodextrin. Depletion and enrichment of cholesterol using methyl-beta-cyclodextrin is correlated with increased and with decreased acyl-chain flexibility, respectively. Very interestingly, the effects of A β are again connected with the content of cholesterol in modulated SPM. As a reduction of membrane bound cholesterol results in increasing effects of A β , whereas cholesterol enrichment decrease the disturbing properties of A β towards neuronal membranes. In our next step we switched to an in vivo system. We orally treated mice with a lipophilic inhibitor of the cholesterol synthesis. Lovastatin treatment over three weeks diminishes brain membrane cholesterol content by approximately 30 %. However, this reduction of cholesterol levels has no impact on the acyl-chain flexibility. Moreover, cholesterol depletion in vivo does not amplify the membrane disordering effects A β .

We would like to speculate that in vitro modulation with cyclodextrin and in vivo modulation with lovastatin affects different cholesterol pools within the membrane. To test this hypothesis we introduced a special technique which enable us to discriminate effects on different leaflets of the membrane bilayer. Our investigations ruled out that lovastatin affects both leaflets in SPM gained from statin treated mice: The exo- as well as the cytofacial cholesterol content is significantly reduced by lovastatin treatment. Very interestingly, the statin treatment not only diminishes the levels of cholesterol within the membrane it further varies the transbilayer distribution of cholesterol in these membrane. In contrast in vitro modulation of membrane cholesterol affects exclusively the exofacial leaflet: Depletion using cyclodextrin as well as enrichment using cholesterol-cyclodextrin-inclusion complex decrease and increase only the levels of cholesterol in the exofacial leaflet, respectively.

Taken together, the modulation of membrane cholesterol in vitro affects predominantly the exofacial leaflet of the bilayer. This variation leads to obvious changes in membrane structure which most probably favors the membrane disordering effects of A β on cholesterol depleted membranes. The manipulation of the cholesterol content in vivo

using lovastatin occurs in connection with the very complex cellular cholesterol homeostasis. Thus the cell is able to compensate possible unphysiological cholesterol distributions which probably favours the effects of A β on cellular membranes. We would like to speculate that the intervention with statins does not hold the risk of strengthened disrupting A β effects towards neuronal membranes.

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Lecture 7

BRAIN CHOLESTEROL, STATINS, AND ALZHEIMER'S DISEASE

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Findings that cholesterol regulates cellular APP processing and that treatment of patients with hypercholesterinemia with statins (cholesterol lowering drugs) reduces the risk of Alzheimer's disease (AD) have suggested that brain cholesterol may play an important role in this neurodegenerative disorder. However, the relationships between brain cholesterol, statins, and AD is more complicated than originally thought. Firstly, there is no major change of brain cholesterol in AD and reductions of brain A β levels by statins have been seen under conditions where brain cholesterol was not altered to a relevant degree. Secondly, although same statins (lovastatin, simvastatin) reduce brain cholesterol in animals and man, pravastatin does not. This is not surprising, since pravastatin does not cross, the blood brain-

barrier. However, pravastatin does reduce the AD risk in patients.

These divergent observations might be fitted together by our recent findings that all three statins mentioned change the transbilayer distribution of cholesterol and probably also the raft distribution in mice. This is also the case for pravastatin, suggesting a possible indirect mechanisms. We suggest that bilayer distribution rather than total changes of cholesterol might explain the beneficial effects of statins in AD and might even play a more general role in the disease.

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Lecture 8

CHOLESTEROL AND AMYLOID PRECURSOR PROTEIN METABOLISM

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Research from various fields of Alzheimer's disease (AD) research has confirmed in the very recent years a role of cholesterol in AD. Retrospective epidemiological studies show dramatic difference in AD statin users and non-users. *In vivo* cholesterol feeding increases plaque formation and inhibition of cholesterol synthesis reduces cerebral A β 40 and A β 42 production. Clinical studies verify this concept since statins reduced A β blood levels and very recently we could show that Simvastatin at 80mg/day reduces cerebrospinal fluid A β levels significantly after 6 month of treatment. From a cell biological point of view our results show a new and completely unexpected link between cholesterol, cholesterol trafficking and the regulation of enzymatic activity central to AD.

On the cellular level A β production depends on the enzymatic cleavage by APP-secretases. When

deciphering the molecular mechanisms involved, we found that sub-cellular cellular lipid composition and lipid trafficking regulate the balance between the amyloidogenic and the non-amyloidogenic pathway. Lipid targeted treatments revealed a complex lipid dependent regulation of APP secretases in neurons. (1) β -secretase activity is reduced upon cholesterol depletion, as is γ -secretase activity. Remarkably, these secretases have little in common; still the same cholesterol lowering treatments inactivates them. Moreover, these effects are additive to each other, thus a small inactivation of both enzymes results in a pronounced loss of A β , as revealed by cholesterol manipulations restricted to different sub-cellular compartments. (2) This raises the possibility that cellular cholesterol trafficking is involved in A β generation. LDL derived cholesterol uptake is critically dependent on the function of the NPC1 protein localized to a late endosomal compartment. Exposure of neuronal cells to cholesterol transport / NPC1 function -inhibiting agents (Imipramine or U18666A) resulted in decreased β -secretase activity. In contrast, γ -secretase activity was enhanced, increasing the production of A β 40 and A β 42. These experiments illustrate the independent and mechanistically separate nature cholesterol treatments have on the different constituents of the A β generating molecular machinery.

Blocking the intracellular cholesterol trafficking at the level of late endosomes also resulted in a parallel increase of cholesterol, presenilin (PS) and A β 42 in specific vesicles relevant for cholesterol trafficking. The unexpected colocalization of certain ER markers indicates that block of cholesterol trafficking prevented export of APP-CTFs and PS from these vesicles. Moreover, it indicates that under these conditions γ -secretase is active in a compartment previously thought to play a role only in cholesterol trafficking. PS is mainly localized distant from sites of secretase activity. Transient fusion of these vesicles may help to understand this spatial paradox.

Taken together these data provide a rational cellular mechanism explaining the epidemiological and prospective clinical data suggesting a beneficial role of low cholesterol levels in AD or dementia in general. Furthermore, our data provide evidence that reduced A β production may not be achieved by reducing the mean cholesterol content of neurons, but rather by alterations in cellular cholesterol distribution, uptake and storage.

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Lecture 9

CHOLESTEROL IN SIGNAL TRANSDUCTION AND APP METABOLISM

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The amyloid precursor protein is cleaved in the secretory, non-amyloidogenic pathway by α -secretases within the sequence of the amyloid β -peptides, thereby releasing a neuroprotective fragment (APPs- α). It has been shown that the disintegrin metalloprotease ADAM 10 has basal and protein kinase C-stimulated α -secretase activity, and many properties expected for a physiologically relevant α -secretase.¹ Conditions by which the α -secretase activity can be enhanced might be beneficial for the treatment of Alzheimer's disease.

One approach to enhance the α -secretase activity is the activation of G-protein coupled receptors that are linked to phospholipase C and Ca²⁺ mobilization: pharmacological activation of muscarinic m1-receptors has recently been shown to decrease the level of total A β in CSF of patients suffering from AD.²

Another approach is the reduction of cellular cholesterol which stimulates the non-amyloidogenic pathway by its effect on the α -secretase ADAM 10. Treatment of various peripheral and neural cell lines with either the cholesterol-extracting agent methyl- β -cyclodextrin or the hydroxymethyl glutaryl-CoA (HMG-CoA) reductase inhibitor lovastatin resulted in a drastic increase of secreted α -secretase-cleaved, soluble APP. In cells overexpressing APP, the increase of α -secretase activity resulted in a decreased secretion of A β

peptides. Several mechanisms were elucidated as being the basis of enhanced α -secretase activity: impaired internalization of APP was responsible for the effect observed with methyl- β -cyclodextrin; treatment with lovastatin resulted in higher expression of the α -secretase ADAM 10.³

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Lecture 10 (was not delivered as authors could not attend)

24S-HYDROXYCHOLESTEROL: A MARKER OF BRAIN CHOLESTEROL METABOLISM

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The enzymatic conversion of CNS cholesterol to 24S-hydroxycholesterol, which readily crosses the blood-brain-barrier, is the major pathway for elimination of brain cholesterol and the maintenance of brain cholesterol homeostasis. The enzyme mediating this conversion has been characterized at the molecular level (CYP46), but the mechanisms of its regulation are not yet known. Like other oxysterols, 24S-hydroxycholesterol is efficiently converted into normal bile acids or excreted into the bile in its sulphated and glucuronidated form. The levels of 24S-hydroxycholesterol in the circulation decrease with age in infants and children. In adults, however, the levels appear to be stable.

There is accumulating evidence pointing toward a potentially important link between cholesterol, β -amyloid, and Alzheimer disease. Plasma concentrations of 24S-hydroxycholesterol from

Alzheimer and vascular demented patients are significantly higher compared with healthy subjects. Variations in genetic background, time of disease onset, and severity of dementia are potential sources of variance. Measurement of 24S-hydroxycholesterol levels in the cerebrospinal fluid may provide more accurate data describing the progression of neurodegeneration.

Inhibitors of cholesterol biosynthesis, also termed statins, seem to have a reductive influence on the generation of the amyloid precursor protein, the neuronal secretion of β -amyloid, and on cholesterol de novo synthesis. Recent epidemiological studies indicate that the prevalence of diagnosed AD and vascular dementia is reduced among people taking statins for a longer period of time. High-dose simvastatin treatment (80 mg/day) in patients with hypercholesterolemia leads to a significant decrease of serum concentrations of brain-specific 24S-hydroxycholesterol and indicates a diminished cholesterol metabolism in the brain. Treatment with high-dose simvastatin in normocholesterolemic Alzheimer patients at early stages of the disease for 26 weeks results in a significant decrease of A β -levels in cerebrospinal fluid. This decrease correlates with the reduction of 24S-hydroxycholesterol.

We conclude that high-dose simvastatin treatment in early stages of Alzheimer disease may result in delay of the pathogenesis of β -amyloid, reasoned by a lowering of brain cholesterol metabolism.

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POSTER PRESENTATIONS

Poster 1

EXPRESSION OF LOW DENSITY LIPOPROTEIN RECEPTOR REGULATES PHOSPHOLIPID MOLECULAR SPECIES IN BRAIN SYNAPTIC PLASMA MEMBRANES

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Several different lines of research (e.g., apoE4, cholesterol, ethanolamine plasmalogen) support the importance of lipid dynamics in Alzheimer's disease (AD) pathogenesis. However, an understanding of the mechanisms of these various lipid mediators is not well-understood. The low density lipoprotein receptor (LDLR) is one of the receptors that bind apoE and plays a major role in cell lipid homeostasis. In addition, to its role in cholesterol regulation, evidence using non-neuronal tissue indicates that the LDLR indeed may be involved in phospholipid and fatty acid homeostasis particularly with respect to unsaturated fatty acids. In the present study, the phospholipid molecular species of synaptic plasma membranes (SPM) of control and LDLR-deficient mice were examined. The phospholipid molecular species of phosphatidylcholine (PC), diacyl-phosphatidyl-ethanolamine (diacyl-PE), alkenylacyl-PE, alkyl-acyl-PE, phosphatidylserine (PS), and phosphatidylinositol (PI) of SPM were determined using reverse-phase HPLC/electrospray ionization mass spectrometry (ESI-MS). Each phospholipid class displayed a distinct molecular species distribution and sizable differences were observed in PS and alkylacyl-PE molecular species between LDLR-deficient mice and control mice. Both PS and alkylacyl-PE were significantly reduced in LDLR-deficient mice compared with control mice. Of special interest were data showing that phospholipids with polyunsaturated fatty acids in the sn-1 or sn-2 position, or both positions were significantly reduced in SPM of LDLR-deficient mice. Alterations in LDLR structure or its ability to

bind lipoproteins may disrupt endocytosis and recycling of lipids to the plasma membrane and intracellular organelles. Results revealed a new role for LDLR in regulating phospholipid molecular species in neuronal membranes.

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Poster 2

STATINS INDUCE ALTERATIONS IN SPM TRANSBLAYER CHOLESTEROL DISTRIBUTION: NEW PHARMACOLOGICAL INSIGHT FOR THE PREVENTION OF ALZHEIMER'S DISEASE?

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Information of the pharmacological effects of statins on the CNS is clearly lacking. Actually, these potent inhibitors of the HMG-CoA reductase undergo some kind of renaissance since they are discussed to exert positive effects in the pathogenesis of AD by possibly affecting the amyloidogenic processing of intracellular APP. This interference is probably linked to cellular cholesterol homeostasis. Recently we could demonstrate that *in vivo* treatment of mice with the lipophilic compound lovastatin resulted in a discrete reduction of brain membrane cholesterol levels.¹ To follow up these insights, we subsequently performed *in vivo* studies including lovastatin and simvastatin as lipophilic agents as well as pravastatin as a hydrophilic compound, focussing on their efficiency to affect subcellular membrane cholesterol pools in the brain of mice. The statins exert different effects on the cholesterol homeostasis in brain synaptic plasma membranes. In contrast to the hydrophilic pravastatin, the lipophilic lovastatin and simvastatin strongly reduce the levels of free cholesterol in SPM. The statins change the leaflet distribution of cholesterol in SPM and reduce the expression of the raft marker protein flotillin. Lovastatin and pravastatin but not simvastatin reduce cholesterol levels in the exofacial leaflet. These changes are accompanied by modified membrane bulk fluidity. Very interestingly, these variations are only observed for substances with clinical efficiency in AD prevention trials, which points out that alterations in

transbilayer cholesterol distribution probably represent the underlying mechanism that forces amyloidogenic processing of APP in AD. Thus, our data contributes to the understanding of the pharmacological mode of action of statins to reduce the prevalence of AD.

Supported by the Hanna Bragard Foundation.

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Poster 3

CHOLESTEROL DIMINISHES MEMBRANE DISTURBING PROPERTIES OF A β IN BRAIN SYNAPTIC PLASMA MEMBRANES OF MICE

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Growing evidence indicates a significant linkage between amyloid-beta protein (A β) and cholesterol metabolism, although the exact role of cholesterol in brain aging and in the pathogenesis of Alzheimer's disease (AD) is still unknown. Recently, *in vitro* and *in vivo* modification of cell cholesterol and its effect on A β -generation became a straight focus in the research of AD. Statins were shown to exert some protective effects in this neurodegenerative disorder. In the present study we discretely modulated cholesterol contents of synaptic plasma membranes (SPMs) from middle-aged mice *in vitro* using methyl- β -cyclodextrin (M β CD) and its cholesterol-inclusion-complexes, respectively. The aim of the study was to investigate whether these modulations resulted in altered physico-chemical membrane properties. Therefore we performed membrane fluidity measurements using two fluorescent dyes labeling different membrane regions. Furthermore, we evaluated the effects of cholesterol modulation on the membrane-disturbing properties of A β . Modulation of membrane cholesterol content was linked to changes in membrane properties. Very interestingly, cholesterol content of *in vitro*

modulated neuronal membranes was negatively correlated with the membrane perturbing effects of A β .

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Poster 4

MEMBRANE RAFT DISRUPTION AT THE BASIS OF AMYLOID DEPOSITION IN ALZHEIMER'S DISEASE PATIENTS WITH APOE4 ALLELE

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The molecular mechanisms underlying the role of ApoE4 as a risk factor in Alzheimer's disease are not well established. In this work we show that the hippocampal membrane of ApoE4 Alzheimer's disease patients has low levels of cholesterol and paucity of raft microdomains. These patients also present a reduced membrane binding of plasminogen and low levels of the amyloid-degrading enzyme plasmin. Since identical deficits occur in hippocampal neurons in culture treated with membrane cholesterol-reducing drugs we propose that defects in membrane composition and organization are at the base of decreased degradation of amyloid peptide in ApoE4 carriers.

These findings suggest that not only the inheritance of the ApoE4 allele but also cholesterol deficits or low plasmin levels should be considered as risk factors for early diagnosis of AD. Approaches based on preventing brain membrane cholesterol loss and/or enhancing brain plasmin activity especially in ApoE4 allele carriers could become valuable therapeutical tools for Alzheimer's disease.

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Poster 5

MTT-REDUCTION AS A VALID TOOL TO DETECT A β MEDIATED CYTOTOXICITY? IMPACT OF CHOLESTEROL

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The LDH- and the MTT assay are established test systems to detect toxicity in cell cultures. Both assays are based on different mechanism: The LDH-assay measures the leakage of cytosolic lactate dehydrogenase throughout the cell and depends on membrane disruption. The MTT assay measures the intracellular formation of a formazan product and usually depends on the metabolic activity of the cell. However, for some conditions it has been shown, that the MTT assay also indicates other biochemical parameters. Various investigations show discrepancies regarding the cytotoxicity of A β evaluated in different assays either based on LDH-leakage or MTT-reduction. Moreover, conflicting data were reported for the role of cholesterol in the prevention of A β -cytotoxicity. The aim of the current study was to evaluate the basis of A β effects in both cytotoxicity assays and the impact of cholesterol thereof.

Many substances reduce cell viability, which could be visualized using the LDH- or MTT-assay. Thereby both test systems reveal qualitatively similar results. In contrast, A β treatment of PC12 cells leads to differing result in the respective assays. While the MTT-assay is sensitive to nanomolar concentrations of A β , only micromolar concentrations of A β cause LDH – leakage in PC12 cells. These findings implicate that the effects of A β established using the MTT-assay are either related to reduced metabolic activity and thus indicate enhanced cytotoxicity or related to an enhanced exocytosis of the MTT-formazan crystals by A β as stated elsewhere. To test the hypothesis that changes in membrane integrity could be displayed by the MTT-assay we modulated the membrane cholesterol content using methyl- β -cyclodextrin inclusion complexes. Next we examined the effects of cholesterol on LDH-leakage and MTT-reduction. Cholesterol has no impact on the release of LDH up to 5 μ M. In this concentration range cholesterol exhibit significant effects in the MTT-assay. However, effects of cholesterol and A β measured by LDH- or MTT-assay are quite similar. Very interestingly, effects on MTT-measurements were significantly correlated with the membrane fluidity of the cells. These findings indicate that the effects of cholesterol observed in the MTT-assay are related to changes of membrane properties. Since we show herein that the effects of A β and cholesterol

measured by MTT-assay are rather related to membrane-based processes than acute cytotoxicity we evaluate possible synergistic effects of both compounds. Cells enriched in cholesterol show a significant reduced susceptibility to A β in the applied concentration range. Microscopic examinations ruled out that cholesterol and A β cause rapid release of MTT-formazan crystals on the exofacial leaflet of the membrane indicating accelerated exocytosis of the test product. Taken together reduced MTT-measurements after A β or cholesterol incubation of cells rather indicate membrane related variations in the endo- and exocytosis of MTT substrate and MTT-formazan crystals, respectively, than enhanced cytotoxicity.

Poster 6

DECREASED MEMBRANE FLUIDITY IN ALZHEIMER'S DISEASE BRAINS

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Alterations in membrane properties are believed to contribute to the neurological disorders observed in Alzheimer's disease. Among several factors, the physical properties of membranes are influenced by the content of cholesterol - which can contribute to the neurological dysfunction in Alzheimer's disease by impairing synaptic transmission - and by oxidative modification of phospholipids - leading to a change in membrane fluidity and thereby affecting synaptic transmission as well as neuronal survival. We wanted to test the hypothesis that membrane fluidity in Alzheimer's disease is decreased and is dependent on levels of cholesterol as well as lipid peroxidation and therefore analysed cholesterol content and malonaldehyde levels as well as membrane fluidity parameters in post-mortem autopsy brain samples from Alzheimer's disease patients and age-matched controls.

The cholesterol content was not different between Alzheimer's disease patients and controls in any of the four brain regions. There was only a tendency towards increased cholesterol content in Alzheimer's disease samples taken from temporal cortex when compared to control samples from the same brain region. Levels of malonaldehyde

were not different between Alzheimer's disease and control samples.

Membrane fluidity in the hydrocarbon core, however, was significantly reduced in frontal and temporal cortex in Alzheimer's disease samples compared to control samples from the same brain region.

Correlation with cholesterol content revealed a positive correlation: with increasing content of cholesterol, membrane fluidity decreases. Correlation with malondialdehyde levels gave no significant results.

Our results show that membrane fluidity is modified by the content of cholesterol and that reduced membrane fluidity can be found in those brain regions that are vulnerable to the pathology of Alzheimer's disease.

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Poster 7

CHOLESTEROL LEVELS INFLUENCE CELLULAR PROCESSING AND TARGETTING OF BETA-SECRETASE

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Epidemiological studies have shown that taking cholesterol-biosynthesis inhibitors (statins) reduces the risk of developing Alzheimer's disease (AD) in the human population by 70%.^{1,2} We have previously shown that increasing cellular cholesterol increases the secretion of the neurotoxic

agents beta-amyloid 1-42 and 1-40 from human cells in culture, whereas reducing cholesterol content by lovastatin treatment dramatically lowers beta-amyloid output.^{3,4} We have now found that increasing cholesterol levels in cells inhibits glycosylation of beta-secretase, the protease that releases the N-terminus of beta-amyloid from its precursor protein (APP), whereas lovastatin promotes its glycosylation, probably by releasing beta-secretase from intracellular cholesterol-rich rafts into other intracellular compartments in which full glycosylation takes place. These effects on intracellular targeting are likely to be the mechanisms of the protective clinical effects of statins.

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Poster 8

UPDATE ON GENETIC ANALYSIS OF LATE-ONSET ALZHEIMER'S DISEASE

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Cholesterol metabolism has been suggested as being implicated in the pathology of Alzheimer's disease (AD), but beside the APOE gene, no other genes have been described as an indisputable risk factor for the disease. However, various genome scans showed linkage between the disease and several chromosomal regions.^{1,2} Therefore we sought to determine whether other gene involved in

the metabolism of cholesterol could mediate the development of AD.

After selecting candidate genes that are located in the regions described as linked to AD, we performed association studies on a case-controls series in order to evaluate their contribution in the evolution of the pathology in the affected subjects compared to the controls. HMG-CoA reductase,³ Lp(a)⁴ and ABCA1⁵ were the first genes selected for this study. Genotyping was conducted on a case-control community-based series composed of 400 individuals affected by the late-onset form of AD, and 400 individuals control without any sign of dementia. Analysis was performed with the standard Pearson Chi-Square or Fisher exact test to evaluate the significance of the genotype distribution. We also evaluated the subgroups according to the APOE genotype.

In the four polymorphisms that were studied in the ABCA1 gene, only the one in the promoter showed a significant association with AD ($p=0.01$), but only in the subgroup carrying at least one APOE2 allele. The T allele frequency for this group was 0.33 in the affected cases and 0.54 in the controls. Likewise, the polymorphism in the Lp(a) gene presented an association with the disease only in the subgroup composed of the APOE2 allele carriers ($p=0.01$). Unlike the first 2 genes, the HMGCoA reductase did not show any association between the polymorphism studied and AD, no matter which subgroups were analyzed. While the number of subjects was low (80/26 and 87/29 controls versus AD cases for ABCA1 and Lp(a), respectively) further studies are needed to confirm these findings.

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The action of statins in brain ischemia (and possibly in Alzheimer's disease, although remaining to be verified) may not be related to cholesterol lowering but to one or more pleiotropic effects.

In fact, statins stimulate NO-synthesis in endothelial cells of vessel walls, including brain vessels, through inhibition of the isoprenylation of proteins repressing the enzyme NO-synthase.

This could explain two difficulties encountered by some contributors to the symposium:

A. Pravastatin is active although does not penetrate inside the brain. The possible reason is that it activates the NO-synthase in the endothelial cells.

B. Statins are able to stimulate NO-synthase in the rodent brain vessels at much lower doses than lipid lowering (2 versus 20-40 mg/kg). - Submitted September 27, 2002

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